The steady-state relation between current and voltage showed no indication of inward rectification regardless of whether Ca^{2+} current was present or whether it was suppressed in the presence of Cd^{2+}. The membrane resistance of gastric myocytes, at potentials between −80 and −20 mV, was 1.5 to 2.0 gigaohms compared to 10 to 15 megohms in guinea pig cardiac myocytes, where the inwardly rectifying K^+ current is well developed (*13*). Hyperpolarizing conditioning clamp pulses also did not activate an inward Na^+ current or reveal a TTX-sensitive component of the Ca^{2+} current. We conclude, therefore, that gastric cells lack a TTX-sensitive Na^+ channel.

Our findings reveal Ca^{2+} and Ca^{2+}-activated K^+ channels in isolated single smooth muscle cells of the stomach. The Ca^{2+}-activated K^+ current was present even though Ca^{2+} was buffered with CaEGTA in the nanomolar range, which may indicate that the activation of this current occurs at a site that excludes EGTA. Ca^{2+} channels in mammalian gastric smooth muscle were effectively blocked by Cd^{2+} and Co^{2+} but appeared to be relatively insensitive to the Ca^{2+} antagonists D600 and diltiazem. This behavior of gastric Ca^{2+} channels is different from that in cardiac ventricular myocytes, in which organic and inorganic Ca^{2+} antagonists are equally effective in blocking the channel (*14*).

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U1 Small Nuclear RNA Genes Are Subject to Dosage Compensation in Mouse Cells

Abstract. *Multiple copies of a gene that encodes human U1 small nuclear RNA were introduced into mouse C127 cells with bovine papilloma virus as the vector. For some recombinant constructions, the human U1 gene copies were maintained extrachromosomally on the viral episome in an unrearranged fashion. The relative abundance of human and mouse U1 small nuclear RNA varied from one cell line to another, but in some lines human U1 RNA accounted for as much as one-third of the total U1. Regardless of the level of human U1 expression, the total amount of U1 RNA (both mouse and human) in each cell line was nearly the same relative to endogenous mouse 5S or U2 RNA. This result was obtained whether measurements were made of total cellular U1 or of only the U1 in small nuclear ribonucleoprotein particles that could be precipitated with antibody directed against the Sm antigen. The data suggest that the multigene families encoding mammalian U1 RNA are subject to some form of dosage compensation.*

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The U class of small nuclear RNA's (U snRNA's) is found in all eukaryotic cells and has been highly conserved during evolution. The U snRNA's range in size from 60 to 216 nucleotides, have a trimethylguanosine cap structure at the 5′ end (with the exception of U6), have highly modified nucleotides, and are not polyadenylated. Most are rich in uridine. The sensitivity of U1 and U2 synthesis to α-amanitin and to DRB (5, 6-dichloro-1-β-D-ribofuranosyl benzimidazole) suggests that these snRNA's are products of RNA polymerase II (*1*). Most, if not all, snRNA's are associated with seven or more proteins in the form of distinct small nuclear ribonucleoprotein particles (snRNP's) (*2*). The study of snRNP structure and function has advanced dramatically since the discovery that autoimmune antibodies in disorders such as systemic lupus erythematosus and polymyositis are often directed against specific snRNP proteins. The U1 snRNP recognizes the 5′ splice junction in mRNA precursors, and the other species of snRNP may also be important in nuclear RNA processing (*3*). The genes for the two most abundant snRNA species, U1 and U2, have been isolated from many eukaryotes including humans, rats, mice, chickens, frogs, fruit flies, and sea urchins (*4*). Pseudogenes are especially abundant in the large multigene families encoding human U1 and U2 RNA (*5*). The human U2 genes are organized in a large tandem array with a 6-kilobase (kb) repeat unit and are clustered at a single site on chromosome 17 (*6*). The organization of the U1 genes may be very similar (*6*). The upstream sequences that promote transcription of U1 and U2 snRNA by RNA polymerase II appear to be different from elements responsible for the initiation of most mRNA species (*7*).

To better understand the expression and regulation of human U1 genes in vivo, we cloned human U1 genes into a vector derived from bovine papilloma virus and introduced the recombinant constructions into C127 mouse cells (*8*). Lund *et al.* (*6*) has shown that human U1 genes can be expressed in mouse cells. Bovine papilloma virus (BPV-1), or a fragment (69 percent) of the viral genome, can transform various susceptible rodent cells that are nonpermissive for viral packaging. Transformed cell lines can be propagated indefinitely and contain multiple copies of the viral DNA as extrachromosomal circular molecules (*9*). When the BPV genome is fused with a pBR322-derived bacterial plasmid lacking the "poison" sequences that inhibit transformation of eukaryotic cells (*10*), the resulting hybrid replicon is capable of shuttling back and forth between bacterial and mammalian cells.

Two human U1 genes (HSD2 and HSD4) isolated by Manser and Gesteland (*4*) and two U2 full-length pseudogenes (U2-18 and U2-19) previously described (*5*) were inserted into the BPV vector 240-6 (Fig. 1). This vector is a derivative of vector 142-6 (*9*), containing the complete BPV genome inserted in a pBR322 derivative, pML2d (*10*). The DNA's were transfected into mouse C127 cells by the calcium phosphate precipitation method (*11*); 3 weeks later, transformed foci were picked, cloned, and thereafter maintained as cell lines. The copy number of the episomal BPV recombinants was determined by blotting appropriate restriction enzyme digests of low molecular weight extrachromosomal DNA (*12*) and was characteris-

tic of each cell line, ranging from 10 to 100 copies per cell (Table 1). Only a few cell lines carried the original recombinant constructs in an unrearranged form (Table 1). Rearrangement may be partially dependent on the site at which human U1 genes are inserted into the BPV vector (*13*). In any case, none of these cell lines had chromosomally integrated BPV or human sequences as judged by genomic blotting of high molecular weight chromosomal DNA. As restriction mapping is relatively insensitive to small changes in the DNA, we cannot exclude the possibility that apparently unrearranged extrachromosomal human U1 genes have sustained subtle mutations that could affect episomal copy number, human U1 gene expression, or both. Recent studies suggest that transfected DNA is initially subject to high rates of mutation and rearrangement, perhaps as a consequence of the transfection process itself (*14*). This would be consistent with our data, since no difference in the blotting pattern of Hirt supernatants could be detected when cells that had been frozen in liquid nitrogen were compared to cells that had been passaged 30 to 40 times.

In order to examine the expression of human U1 genes in the cell lines carrying unrearranged DNA, we had to distinguish human U1 snRNA from the endogenous mouse U1 species. We therefore resorted to two very different techniques, two-dimensional oligonucleotide fingerprinting and native gel electrophoresis, because the sequence of mouse U1 RNA has not yet been determined. Initially, we compared the T1 ribonuclease fingerprints of purified U1 RNA from HeLa cells (Fig. 2A) and mouse C127 cells (Fig. 2B); the latter express only U1a RNA (*15*). We found a single diagnostic oligonucleotide corresponding to the 3′ end of U1 that migrates reproducibly at different positions in the fingerprints of human and mouse U1 RNA; this nonamer corresponds to spot 17 in the nomenclature of Reddy and Busch (*16*). When total labeled RNA from mouse cell line 3E (which contains human U1 genes from the HSD4 locus) was resolved on a denaturing gel, only one band could be discerned with the mobility of U1 RNA. This band contained both human and mouse U1 RNA, as shown by the presence of both diagnostic 3′ nonamers in the T1 fingerprint (Fig. 2C). Fingerprinting proved to be laborious and, consequently, we sought a simpler and more quantitative assay.

Human and mouse U1 RNA's could be resolved in a single dimension (Fig. 3) by a modification of the native gel electrophoresis system devised by Korn and Gurdon (*17*) for separation of *Xenopus* somatic and oocyte 5*S* ribosomal RNA's. We confirmed by T1 oligonucleotide fingerprinting that the upper band contained exclusively mouse U1 RNA and the lower band exclusively human U1 RNA. With this one-dimensional native gel assay, we found that several different transformed cell lines containing apparently unrearranged human U1 genes expressed both human and mouse U1 RNA (cell lines 1B, 1J, 2A, 3C, 3E, 5I, and 6J; Table 1; Fig. 3, lanes a and b). No human U1 RNA could be detected in cell lines transformed with a human U2 pseudogene (cell lines 7A and 8C; Fig. 3, lanes c and d), in cell lines where the original U1 constructs have sustained obvious rearrangements or deletions, in cell lines transformed with the BPV 240-6 vector alone, or in the untransformed parental C127 mouse cells.

In the independent cell lines express-

Table 1. Characteristics of the transformed cell lines. In all transfection experiments, 2.5×10^5 cells were initially plated. Supercoiled plasmid DNA (0.5 μg) and mouse genomic DNA (10 μg as carrier) formed the calcium phosphate precipitate. After 30 minutes at room temperature, the precipitate was added to the cells and incubated for 4 hours at 37°C. The cells were then treated with 20 percent glycerol for 1 to 2 minutes and grown for at least 3 weeks until foci were large enough to be cloned (*11*).

Gene orientation*	DNA transfected	Cell line	Copy number of BPV episomes	Human U1 as percentage of total U1 RNA
+	BPV-HSD2-4	1B	80	10
		1J*†	10, 50‡	8–10
−	BPV-HSD2-1	2A	10–20	10–15
+	BPV-HSD4-1	3C	10–20	28–33
		3E	8–12	22–25
−	BPV-HSD4-2	4C	100‡	0
+	BPV-D4/Hinc-1	5I	10–20	10–12
−	BPV-D4/Hinc-2	6J*†	15, 80‡	10
+	BPV-U2-18-24	7A	50‡	0
−	BPV-U2-19-72	8C	60–80	0

*A "plus" indicates that the snRNA gene has the same polarity as the BPV early transcription unit; "minus" indicates the opposite orientation. †The cell lines contain a mixed population of episomes. ‡U1 or U2 sequences were deleted from these plasmids.

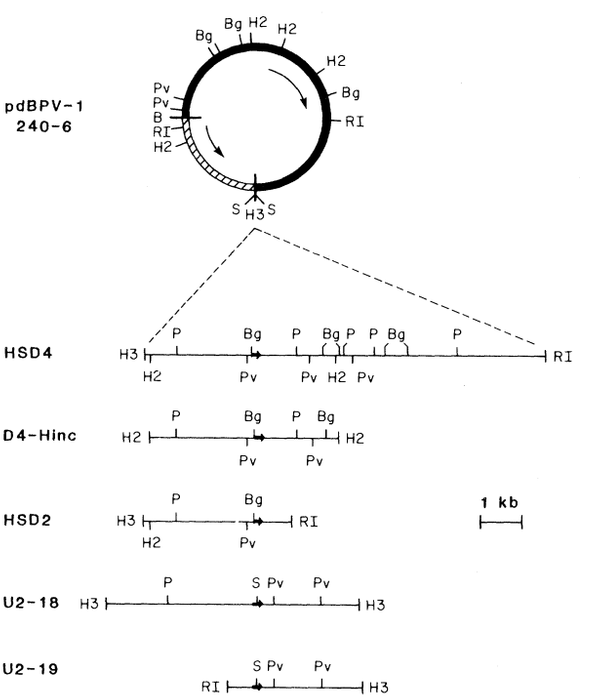

Fig. 1. Structure of the recombinant BPV plasmids. (Top). Physical map of the BPV-1 vector (pdBPV-1 240-6) used in constructing the recombinant clones. The plasmid consists of the complete viral genome (solid line) that was opened at the Bam HI site and cloned into the pML2d vector (hatched line). After destruction of the original BPV-1 Hind III site, one viral Bam HI site was converted into a polylinker containing a Hind III cloning site flanked by two Sal I sites. Arrows indicate the direction of transcription for the β-lactamase gene in pML2d and of the BPV-1 transforming segment. A 59-base-pair (bp) enhancer has been identified (*20*) at the 3′ end of the early polyadenylation signal of BPV-1 genome near the Sal I–Hind III site. (Bottom). Physical map of the human U1 genes and U2 pseudogenes. All fragments were inserted in both orientations into the single Hind III cloning site of pdBPV-1 240-6 (with the 29-bp Hind III–Eco RI fragment of pBR322 as an adapter when necessary) or into the filled-in Sal I site at the same position. U1 and U2 coding regions are indicated with a small arrow (not drawn to scale). Names of the corresponding recombinant plasmids and their orientation are shown on Table 1.

Table 2. Relative expression of U1 RNA in transformed cell lines 3C, 3E, 7A, and 8C. The autoradiogram shown in Fig. 3 was scanned with a GS300 densitometer (Hoefer Scientific Instruments). The peaks were cut out, weighed, and normalized to 5S RNA (lanes a to d) or U2 snRNA (lanes e to h). The tabulated numbers represent an average derived by scanning two different exposures of the autoradiogram (3 and 12 hours). The tabulated numbers are slightly larger for the immunoprecipitated lanes (lanes e to h) compared to the total RNA lanes (lanes a to d) because human U1 snRNA appears to be immunoprecipitated preferentially. The relative precipitability of the different U snRNA's can vary, even in antibody excess, regardless of whether polyclonal human autoantibodies or monoclonal mouse antibodies are used in different cell extracts. For example, compare the amounts of mouse U4 and U5 snRNA in Fig. 3, lanes a to d, with those in lanes e to h. Data derived from densitometry scans were confirmed by scintillation counting of the radioactivity in the excised mouse and human U1 bands and in the mouse U2 doublet.

Transformant	Gene	Mouse 5S	Mouse U2	Mouse U1	Human U1	Total U1	Human U1 as percentage of total U1 RNA
			Total RNA				
3C	HSD4 (human U1)	100		17.8	6.8	24.6	28
3E	HSD4 (human U1)	100		19.2	5.4	24.6	22
7A	U2-18 (U2 pseudogene)	100		22.7		22.7	
8C	U2-19 (U2 pseudogene)	100		23.1		23.1	
			Immunoprecipitated RNA				
3C	HSD4		100	93.9	47	140.9	33
3E	HSD4		100	107.5	35.5	143	25
7A	U2.18		100	135.3		135.3	
8C	U2.19		100	204.5		204.5	

ing human U1 RNA, the level of human U1 relative to total U1 (mouse + human) varied from 10 to 33 percent (Tables 1 and 2 and Fig. 3) as judged both by scintillation counting of excised RNA bands and by densitometry of the corresponding autoradiographs (Fig. 3 and Table 2). The levels of human U1 expression did not correlate with human U1 gene copy number (Table 1). Moreover, the percentage of human U1 was similar whether we examined total cellular U1 (Fig. 3, total RNA lanes) or only the U1 that could be precipitated in the form of U1 snRNP by autoantibodies directed against the Sm antigen (Fig. 3 and Table 2). However, the total amount of human U1 + mouse U1 RNA did not vary significantly from one cell line to another after normalization to endogenous mouse 5S or U2 RNA's (Table 2). Because the relative expression of human and mouse U1 RNA does not greatly affect the total level of U1 RNA in the transformed cells, it would appear that U1 RNA is subject to some form of dosage compensation; that is, the mouse cell appears to regulate the total amount of cellular U1 RNA regardless of gene dosage.

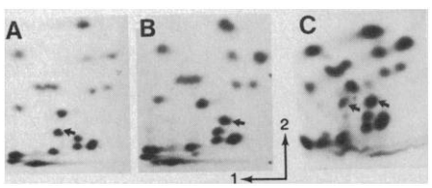

Fig. 2. T1 ribonuclease fingerprints of U1 RNA's immunoprecipitated by antiserum to Sm. Cells were grown to a density of 2×10^5 per milliliter in AutopoM medium plus fetal bovine serum (5 percent) (Flow and Gibco laboratories) and then labeled with orthophosphate (20 μCi/ml) overnight in phosphate-free medium supplemented with dialyzed fetal bovine serum (5 percent). The cells were harvested, washed, sonicated, and immunoprecipitated with antibodies directed against the Sm antigen (*2*). A portion of the sonicate was extracted with phenol directly to provide a sample for total RNA (Fig. 3, lanes a to d). RNA in the immune precipitate was extracted with phenol, resolved on a denaturing 10 percent polyacrylamide gel in 7*M* urea, eluted from the gel, and concentrated by ethanol precipitation. T1 digests were fingerprinted by electrophoresis on cellogel or cellulose acetate at *p*H 3.5 and then subjected to homochromatography on PEI plates with homomix C (*2*). (A) Human U1 RNA from HeLa cells. (B) Mouse U1 RNA from C127 cells. (C) U1 RNA from transformed cell line 3E. Arrows denote spot 17.

We do not yet know whether U1 dosage compensation occurs at the level of U1 transcription, U1 RNA processing, or snRNP assembly. Although we cannot strictly discount the possibility that we selected transformed cells with changes in the copy number of endogenous mouse U1 genes, this is unlikely because transformants containing human U1 genes were obtained at the expected frequency; moreover, we cannot rigorously evaluate this possibility until the structure and organization of the 10 to 20 genes in the mouse U1 multigene family have been characterized in greater detail (*18*). Because the BPV vector system appears to be especially subject to rearrangement, integration, and presumably mutation (*19*, *20*), BPV vectors are not suitable for investigating the mechanism (or mechanisms) of dosage compensation.

Prokaryotes and lower eukaryotes clearly require special regulatory circuits to adapt to changing nutritional and environmental conditions, but complex regulatory circuits are also required for balanced synthesis of the different components in macromolecular assemblies. Al-

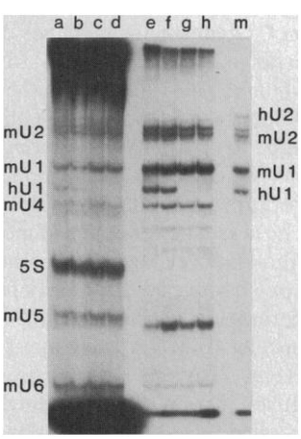

Fig. 3. Separation of human and mouse U1 RNA by native gel electrophoresis. Autoradiogram of a native 12 percent polyacrylamide gel of labeled RNA's immunoprecipitated with an excess of mouse monoclonal antibody (Y12) against Sm antigen. After precipitation, the RNA samples were dissolved in 5 μl of loading solution (10 percent glycerol, 1 m*M* EDTA, 0.05 percent xylene cyanol, and bromophenol blue). The 12 percent gel (ratio of bisacrylamide to acrylamide, 1:20) was prepared in 40 m*M* tris-HCl, 20 m*M* sodium acetate, 2 m*M* EDTA, *p*H 7.8. The electrophoresis buffer was 50 m*M* tris-HCl, 1 m*M* EDTA, 0.4*M* glycine, *p*H 8.3 (*16*). Electrophoresis was performed at room temperature for 7 to 8 hours at constant current so that the gel remained cool. (Lanes a to d) Total nuclear RNA; (lanes e to h) RNA immunoprecipitated by antibody against the Sm antigen. Cell lines: (lanes a and e) 3C; (lanes b and f) 3E; (lanes c and g), 7A; and (lanes d and h) 8C. Purified mouse and human U1 and U2 RNA were markers (lane m).

though the cells of higher eukaryotic organisms generally exist in a comparatively stable environment, they still must balance the synthesis of interacting macromolecules. The tight regulation of globin synthesis by free heme is one example (*21*).

There are two ways in which the RNA and protein components of a ribonucleoprotein complex such as a snRNP could be coregulated. The absolute levels of snRNA might be regulated directly, and snRNA proteins would then be synthesized in sufficient quantity to package all the snRNA. Excess snRNP proteins would either autogenously turn off their own synthesis or simply be degraded. This model may account for the regulation of the components of the bacterial ribosome (*22*). Alternatively, one or more protein components of the snRNP might be regulated directly, and snRNA's would be synthesized until excess snRNA failed to be properly packaged. In this model, excess snRNA would autogenously regulate its own synthesis or be degraded.

The existence of proteins that are common to different snRNP species necessarily complicates any model for regulation of the protein and RNA components of the particles. For example, the Sm protein is common to distinct snRNP's containing U1, U2, U4, U5, U6, and U7; the RNP antigen is restricted to the U1 snRNP (*1*, *2*). The regulatory events responsible for U1 dosage compensation do not seem to affect the other snRNP's: the ratio of total U1 to U2 snRNA in the mouse cell was not increased by addition of human U1 genes. Thus, the second model, which in its simplest form predicts an imbalance among the different snRNA species, cannot account for our data. More complex regulatory circuits might combine features of both models. Although we have shown only that mammalian cells regulate the total level of U1 RNA, it is reasonable to suggest that this will be the case for other snRNA's as well.

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15. Lerner and Steitz (*3*) have shown that the mouse genome encodes at least two distinct species of U1 RNA, Ula and Ulb. The proportion of Ulb to Ula varies from one cell line to another, and terminally differentiated mouse tissues do not seem to express Ulb (S. Wolin and J. A. Steitz, personal communication; E. Lund and J. E. Dahlberg, personal communication). Lund *et al.* (*6*) have shown that mouse C127 cells express no detectable Ulb, although some mouse-human hybrid cell lines express both Ula and Ulb.
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Presence of Laminin Receptors in *Staphylococcus aureus*

Abstract. *A characteristic feature of infection by* Staphylococcus aureus *is bloodstream invasion and widespread metastatic abscess formation. The ability to extravasate, which entails crossing the vascular basement membrane, appears to be critical for the organism's pathogenicity. Extravasation by normal and neoplastic mammalian cells has been correlated with the presence of specific cell surface receptors for the basement membrane glycoprotein laminin. Similar laminin receptors were found in* Staphylococcus aureus *but not in* Staphylococcus epidermidis, *a noninvasive pathogen. There were about 100 binding sites per cell, with an apparent binding affinity of 2.9 nanomolar. The molecular weight of the receptor was 50,000 and* p*I was 4.2. Eukaryotic laminin receptors were visualized by means of the binding of* S. aureus *in the presence of laminin. Prokaryotic and eukaryotic invasive cells might utilize similar, if not identical, mechanisms for invasion.*

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Adhesion is an important prerequisite for bacterial infectivity. Specific molecules are involved in bacterial adhesion at both the bacterial and host cell surfaces (*1*). The role of laminin, the major glycoprotein of the basement membrane, in the adhesion of pathogens such as bacteria has not been clearly established. Laminin is a noncollagenous, high molecular weight (10^6) protein composed of polypeptide chains with molecular weights of approximately 200,000 and 400,000 (*2*). It interacts with glycosaminoglycans and promotes adhesion of various cell types (*3*). Laminin receptors occur on cells that normally interact with basement membranes (*4*) as well as on cells that extravasate, such as metastasizing cancer cells (*5*), macrophages (*6*), and leukocytes (*7*).

Once a devastating pathogen, with over 80 percent mortality, *Staphylococcus aureus* is now the most common cause of severe infection in the nonimmunocompromised patient. In the industrialized world, it is responsible for endocarditis, osteomyelitis, arthritis, soft tissue infection, and pneumonia. In underdeveloped countries, staphylococcal infection is even more serious, with untreated disease often leading to fatal bacteremia (*8*).

Staphylococcus epidermidis is considered to be a nonpathogenic organism (*9*); it can, however, be an important pathogen when delivered to the actual site of infection by prosthetic devices or intravascular catheters (*10*). *Staphylococcus aureus* is able to colonize quickly and invade through minor breaks in skin and mucous membranes and reach the bloodstream. Once bloodborne, *S. aureus* can cause acute endocarditis and widespread metastatic abscesses (*11*). We now report the presence of laminin receptors on *S. aureus* (Cowan I strain), but not on *S. epidermidis*.